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Bouldering: an alternative strategy to long-vertical climbing in root-climbing hortensias

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In the Neotropics, the genus *Hydrangea* of the popular ornamental hortensia family is represented by climbing species that strongly cling to their support surface by means of adhesive roots closely positioned along specialized anchoring stems. These root-climbing hortensia species belong to the nearly exclusive American *Hydrangea* section *Cornidia* and generally are long lianescent climbers that mostly flower and fructify high in the host tree canopy. The Mexican species *Hydrangea seemannii*, however, encompasses not only long lianescent climbers of large vertical rock walls and coniferous trees, but also short 'shrub-like' climbers on small rounded boulders. To investigate growth form plasticity in root-climbing hortensia species, we tested the hypothesis that support variability (e.g. differences in size and shape) promotes plastic responses observable at the mechanical, structural and anatomical level. Stem bending properties, architectural axis categorization, tissue organization and wood density were compared between boulder and long-vertical tree-climbers of *H. seemannii*. For comparison, the mechanical patterns of a closely related, strictly long-vertical tree-climbing species were investigated. *Hydrangea seemannii* has fine-tuned morphological, mechanical and anatomical responses to support variability suggesting the presence of two alternative root-climbing strategies that are optimized for their particular environmental conditions. Our results suggest that variation of some stem anatomical traits provides a buffering effect that regulates the mechanical and hydraulic demands of two distinct plant architectures. The adaptive value of observed plastic responses and the importance of considering growth form plasticity in evolutionary and conservation studies are discussed.

1. Introduction

Climbing plants show a wide range of highly specialized anchoring structures such as hooks, spines, tendrils, twining stems and adhesive roots that allow them to climb on a range of natural and artificial surfaces [1,2]. Different attachment methods provide different degrees of anchoring and confer particular mechanical properties to the plant growth form and life history [2,3]. In general,

climbing plants are characterized by a decrease in stiffness during plant development (e.g. with increasing stem diameter), unlike for instance shrubs and trees that increase their stiffness towards older developmental stages [4]. Climbing plants that ascend by means of tendrils, twining stems and adhesive roots are often more firmly attached than for example those employing hooks or spines. Firmly attached plants usually develop more compliant tissues in older developmental stages than weakly attached ones; this is believed to be advantageous when coping with the potential stress produced by close contact with their supports (e.g. torsion, tensile or shear stress when a host tree swings with the wind [1,2]).

Root-climbers are plants that strongly anchor to their climbing surface by means of adhesive roots. These are either limited to certain positions along the stem, such as in the cheese plant (*Monstera deliciosa*), or all along the stem, as in the English ivy (*Hedera helix*). Root-climbers are also present in the popular ornamental hortensia family (Hydrangeaceae). Within this family, the root-climbing habit has independently evolved in the genera *Decumaria*, *Hydrangea*, *Pileostegia* and *Schizophragma* [5]. Root-climbing *Hydrangea* species almost entirely belong to the nearly exclusive American *Hydrangea* section *Cornidia* ([5], hereafter shortened to *Cornidia* [6]). Adhesive roots in *Cornidia* species are closely positioned along one side of specialized anchoring stems, firmly attaching them to their support surfaces. During extensive fieldwork throughout the Neotropical distribution area of *Cornidia*, which ranges from North Mexico to central-south Chile and Argentina, we have never observed fully self-supporting juvenile or adult individuals. In general, mature individuals of *Cornidia* are long, lianescent climbers developing a major climbing portion, which is commonly secured to tree trunks and more rarely to tall vertical rock walls. Some *Cornidia* species, however, produce a robust self-supporting apical portion giving a 'shrub-like' appearance when climbing on short rounded boulders.

Phenotypic plasticity is the ability to express alternative phenotypes by a single genotype in response to different environmental conditions [7]. Exogenous conditions such as light incidence, forest openness, as well as availability and variability of supports are known to modulate climbing plant development [1,8–10]. Several climbing species vary according to the availability of supports, alternatively growing as self- or non-self-supporting individuals [1,8–13]. Studies combining plant biomechanics and anatomy offer a comprehensive method for characterizing growth form variation [3,4,14–18]. Plant architecture, moreover, provides a powerful method to assess endogenous developmental processes and isolate them from changes due to phenotypic plasticity triggered by environmental conditions [19,20].

Here, we investigate to what extent differences in support size and shape promote growth form plasticity in *Cornidia* species. We focus on the adult phase of development when many lianas experience a conspicuous shift in growth behaviour when reaching the canopy [12,13]. This change in growth behaviour is promoted by a series of covarying environmental conditions including loss of physical support and increased light exposure. Comparing long-vertical climbing surfaces (trees) with shorter rounded ones (boulders) could thus provide a reference framework to which growth form plasticity could be contrasted. Early increased light exposure and premature loss of physical support could lead to structural, mechanical and anatomical plastic responses.

We study *Hydrangea seemannii*, the only temperate *Cornidia* species of the northern hemisphere, which has frequently been reported in herbarium records both as a liana and a shrub. The species exhibits two contrasting climbing phenotypes: (i) long-vertical lianescent climbers on both coniferous trees (e.g. *Cupressus* and *Pseudotsuga*) as well as high vertical rock walls and (ii) short boulder-climbers that are basally anchored to small- or medium-sized boulders producing a 'shrub-like' form that protrudes well above the boulder surface. In order to address plastic responses to support variability in *H. seemannii* (e.g. differences in size and shape), we contrasted the architectural construction, stem bending properties, wood density and tissue organization of its climbing phenotypes. For comparison, mechanical properties of a second, strictly long-vertical tree-climbing *Cornidia* species were investigated. This species is new to science and is herein referred to as *Hydrangea* sp. 1. In contrast to *H. seemannii* and although rock outcrops are present in its habitat, *Hydrangea* sp. 1 climbs exclusively on trees.

Our study aimed to address three specific questions. First, do boulder-climbers and long-vertical tree-climbers of *H. seemannii* differ in their morphological, mechanical and anatomical architectures? If so, can this be considered growth form plasticity resulting from variability in support type? Second, what are the architectural, mechanical and anatomical traits underlying this plasticity? And third, how do mechanical properties of a taxon with 'high' phenotypic plasticity and climbing via both support types differ from the properties characterizing a taxon with a 'low' phenotypic plasticity and restricted to one type of climbing support?

2. Material and methods

2.1. Sampling and habitat description

Specimens of *H. seemannii* were collected in the Santa Bárbara canyon (Pueblo Nuevo, Durango) located in the northern Sierra Madre Occidental of Mexico (1050–2750 m elevation). This locality is a temperate sub-humid coniferous forest where *H. seemannii* is locally abundant and the only lianescent representative [21]. Frosts and occasional snowfalls occur in winter (annual mean temperature: 11.5°C, maximum 21.1°C, minimum 1.9°C; annual mean precipitation: 937.7 mm; 1951–2010). The Santa Bárbara locality comprises a relatively flat area at the top of a canyon and includes a complex range of habitats with variously sized igneous rock outcrops—including long-vertical rock walls and boulders, small waterfalls, perennial streams and a forest understory densely covered by a thick layer of moss. In total, four mature individuals, two per climbing phenotype, were sampled for mechanical, anatomical and architectural axis characterization.

Three specimens of the strictly long-vertical tree-climbing *Hydrangea* sp. 1 were collected from two different localities in the municipalities of San Andrés Tuxtla and Soteapan located in the Sierra de Los Tuxtlas, an isolated volcanic mountain range along the southeastern coast of the Gulf of Mexico in the state of Veracruz. The first of these localities is an extremely humid cloud forest with north-exposed slopes in the Sierra de Santa Marta—a mountain range of volcanic origin (*ca* 1300 m elevation). The second locality is located in a relatively flat rainforest area (*ca* 1050 m elevation) dominated among others by Lauraceae tree species (e.g. *Persea* and *Nectandra*). The *Hydrangea* sp. 1 collection zones are characterized by an average annual mean temperature of 24.3°C (maximum 28.4°C and minimum 20.2°C) and an average annual mean precipitation of 4183 mm (1951–2010). In contrast

to the *H. seemannii* habitat, the tropical forests of this region are characterized by numerous climbing taxa [22].

Owing to their root-climbing nature, portions of selected individuals were carefully detached by hand and cut at their base. Shoots growing along the forest floor and rhizomes were not collected. Higher portions of tree-climbing individuals were not accessible due to their high position on the supporting tree canopy. Although numerous localities of *H. seemannii* are known and despite its local abundance, it has been suggested to be at risk because of habitat destruction, timber extraction and climate change [23]. In addition, the habitat of *Hydrangea* sp. 1 is being continuously destroyed by human activities or local fires suggesting that the species is critically endangered (own observations). As biomechanical and anatomical measurements are necessarily destructive, only a minimal number of stems were sampled after careful evaluation of the local abundance of each species and in consideration of their ability to re-sprout from rhizomes or shoots present on the forest floor.

2.2. Architectural analysis

Plant axes of *H. seemannii* individuals were described morphologically following the revised architectural concepts and methods of Barthélémy & Caraglio [19]. All stems of each sampled individual were categorized in terms of: (1) axis habit, whether the stem was clinging on trees or boulders (climbing), or standing independent of any surface (self-supporting); (2) branching pattern, considered as rhythmic when the stem branches were distributed in tiers or not branched; (3) growth direction, whether stems' growth direction followed (a) that of the contact surface (thigmotropic), (b) was generally vertical (orthotropic), (c) was horizontal (plagiotropic), (d) was mixed with a thigmotropic proximal portion and a plagiotropic distal end or (e) without precise growth direction; (4) presence or absence of adhesive roots; (5) ability to flower; (6) symmetry, whether the leaves and branches of a stem were disposed in all spatial directions (radial) or in one plane (bilateral); and (7) relative growth unit length. Number of axis categories was defined by differences in the above-mentioned morphological features and their function as supporting and/or resource acquisition structures. Different axis types might arise from the same node [19], therefore, distribution of axis categories may not equal branching order.

2.3. Bending tests and calculation of Young's modulus

Individuals of both *Hydrangea* species were subjected to three-point-bending tests where stem segments are positioned on two supporting points. According to the stem segment size, the distance between the two supporting points was adjusted and a bending force was applied in the middle (third point). Bending tests were performed with a portable Instron In-Spec 2200 (Instron, Norwood, MA, USA) using two different force transducers with maximum allowed force of 10 and 250 N, according to sample size and rigidity. Test settings consisted of 0.25 mm s^{-1} of cross-head speed and 1–2 mm of deflection. In order to reduce the influence of shear forces [24], only segments with a suitable span-to-depth ratio of more than or equal to 19 were analysed [16].

Flexural rigidity EI (N mm^2) is the ability of a structure to resist bending in terms of size, geometry and material properties. The higher the flexural rigidity, the stiffer the structure. It was calculated from the formula $EI = (L^3 \times b)/48$, where L (mm) is the distance between the two supporting points and b the slope derived from the force (N) deflection (mm) curve. The second moment of area I (mm^4) is used to quantify the form and size of a segment's cross section relative to the neutral plane of bending. The second moment of area was calculated with the formula $I = (\pi/4) \times a^3 \times b$, where a represents the vertical radius in the bending direction and b the radius perpendicular to a. Young's modulus E (MPa) is a parameter that describes the relation between the segment's stress and strain and quantifies segment's stiffness. The higher the Young's modulus, the higher the resistance of a structure to deformation. This parameter is particularly valuable for the study of stem mechanical properties as it is independent of the segments' size and geometry. Furthermore, Young's modulus can be calculated from the formula $E = EI/I$ [2,4,16,17]. As plant stems are composed of different tissues, Young's moduli of stem segments were interpreted as bulk moduli of the compound stem structure [18].

Stem segments were not debarked but if present, adhesive roots were removed by cutting parallel and close to the stem surface. Measured segments represented all architectural axis categories except for the most distal axis (C5 axes described below), because they lacked appropriate span-to-depth ratios. Similarly, the number of large-diameter axes for boulder-climbers was limited because of their irregular and curved geometries resulting from climbing on the boulder surface.

2.4. Stem tissue organization

Seventeen to 18 segments per climbing phenotype were selected among mechanically tested segments and processed for anatomical observations. These segments were sectioned transversely with a vibratome (Microtome Hyrax-V50, Carl Zeiss Micro-Imaging GmbH Jena, Germany) or with a standard sliding wood microtome. Sections (25–140 µm thick) were stained with carmine green or toluidine blue for distinguishing lignified from non-lignified tissues [25] and photographed with a digital camera (Olympus-DP71) mounted on a transmitted light compound microscope (Olympus-BX51) or a dissecting microscope (Olympus-SZX9). Tissue transverse-sectional area outlines were analysed with the software package ImageJ v. 1.46r [26].

We differentiated three stem tissue categories: (i) parenchymatous pith, (ii) lignified pith along with wood and (iii) soft outer cortical tissues including cortex, secondary phloem and periderm. Then we determined the contribution of each tissue category to the total transverse-sectional area and second moment of area. A formula for an elliptical second moment of area $I = (\pi/4) \times (a^3) \times (b)$ was applied for the parenchymatous pith as for the total transverse-section (see above), and a formula for elliptical rings $I = (\pi/4) \times [(A^3 \times B) - (a^3 \times b)]$ was used for wood along with lignified pith and outer cortical soft tissue rings (electronic supplementary material, figure S1). Additionally, we estimated the degree of pith eccentricity using the parenchymatous pith's geometric centre as the centre of area for the total transverse-section and the ellipses formed by the wood along with pith and the pith itself. Pith eccentricity was estimated by the distance between the parenchymatous pith and transverse-section's geometric centres (α and β, respectively), expressed as a percentage of the mean transverse-section's radius (electronic supplementary material, figure S1).

2.5. Stem developmental stages

Biomechanical and anatomical transitions at different stages of development were used to explore the changes underlying growth form variability [9,18]. Here, we identify two developmental stages of the stem distinguished by a specific anatomical change: a marked shift of the contribution of lignified pith along with wood and parenchymatous pith to the total cross-sectional area and second moment of area. This occurs when stems reach an approximate diameter of 7.4 mm (see the electronic supplementary material, figure S2).

2.6. Wood densitometry

Wood densitometry scans were performed with the Nanowood scanner [27] at the Centre for X-ray Tomography of Ghent University (UGCT, http://www.ugct.ugent.be). Ten segments above

Table 1. Summary of morphological features defining the five axis types composing *H. seemannii* architectural organization. These five axis types are classified in the functional categories for structural support and resource acquisition.

	structural support		resource acquisition		
	C1	C2	C3	C4	C5
axis habit	climbing/self-supporting	climbing	self-supporting	self-supporting	self-supporting
branching pattern	rhythmic	rhythmic	rhythmic	rhythmic	not branched
growth direction	thigmotropic/plagiotropic	thigmotropic	orthotropic	plagiotropic	no precise growth direction
adhesive roots	present/absent	present	absent	absent	absent
flowering ability	no	no	Yes	yes	no
symmetry	bilateral	bilateral	radial	bilateral	bilateral
growth unit length	$\leq$5 m	$\leq$1 m	$\leq$50 cm	$\leq$25 cm	$\leq$5 cm

7.4 mm in diameter were selected, i.e. five segments for each climbing phenotype. One disc of uniform thickness of 0.5 cm was sectioned per segment, gradually dehydrated in a series of alcohol and subsequently air dried. Two helical scans (five discs per scan) were performed at 60 kV and 90 mA scanning for approximately 1 h with a rotation step size of 0.36°. Reconstructions were performed with Octopus, a tomography reconstruction package for parallel, cone-beam and helical geometry [28]. The reconstructed greyscale volumes were converted to specific gravity, further referred to as wood density (kg m^{-3}) following the standard protocol adopted from De Ridder *et al.* [29]. The obtained scan resolution was 15 μm $pixel^{-1}$.

2.7. Statistical analyses

Normal distributions of variables and residuals were tested by Shapiro–Wilk tests, assuming normality at significance values more than 0.05. Non-normally distributed variables were normalized by transformation to their base-10 logarithm. All variables were screened for differences between climbing phenotypes of *H. seemannii* through a nested analyses of variance (ANOVA) with climbing phenotype as fixed factor and individuals as a random nested factor within climbing phenotypes (null hypothesis, H0: no difference between climbing phenotypes). The same analysis was used to compare mechanical properties among climbing phenotypes of *H. seemannii* and *Hydrangea* sp. 1 (H0: no differences between cases). Comparisons between climbing phenotypes were performed making distinction between the two stem diameter classes defined above. Null hypotheses in nested ANOVA tests were rejected at $p < 0.05$.

3. Results

3.1. Morphological architecture

The architectural unit of *H. seemannii* is composed of two main axis categories (table 1). The first consists of two axis types (C1 and C2) that together constitute the main anchoring and perennial structure of the plant (figures 1*a*,*c*, 2*a*, 3*a*,*c* and 4). These structural axes are mostly climbing and thigmotropic, with the exception of the apical self-supporting plagiotropic portions of C1 axes (figures 1*c*, 2*a*, 3*a* and 4*a*,*c*,*d*). They branch rhythmically, have a bilateral symmetry and support the remaining axis types (figures 1*a*,*c*, 2*a*, 3*a*,*c* and 4). C2 axes differ from C1 axes in their reduced secondary growth production, shorter lifespan and an entirely climbing habit. The second axis category is composed of three axis types (C3–C5) whose main function is for resource acquisition and/or flowering (figures 1*a*,*c*, 2*a*, 3 and 4). These three axes are entirely self-supporting and completely lack adhesive roots. C3 and C4 axes branch rhythmically and in addition to leaves, they can also expose the inflorescences (figures 1*a*,*c*, 2*a* and 3). By contrast, C5 axes are unbranched and exclusively produce leaves (figure 3). C3 axes differ from any other axis type in their orthotropic growth direction and radial symmetry (figures 1*c*, 2*a* and 3*a*,*b*). C4 axes have a plagiotropic growth direction (figures 1*a*,*c*, 2*a* and 3*c*), whereas C5 has no precise growth direction (figure 3), however, both have a bilateral symmetry.

When climbing on shaded, vertical and long tree trunks and rock walls, *H. seemannii* modules are very long and the structural axes C1 and C2 cling on their support along most of their length and for most of their lifespan (figure 1). Then the main structural C1 axis progressively changes its growth direction by laterally producing plagiotropic self-supporting modules that increase their frequency towards the end of the support (figures 1*c* and 4*a*). In a young tree-climber a main monopodial C1 axis rhythmically branches by mostly producing thigmotropic C2 axes, which distally bear abundant leafy C4 axes (figures 1*a* and 4*b*). At this early phase of plant development, few orthotropic C3 axes are produced (figure 4*b*). Sympodial C1 axes of mature tree-climbing individuals laterally branch by regularly producing leafy C4 axes along most of their length, while orthotropic C3 axes become more abundant towards the apex of the plant (figures 1*c* and 4*a*). Conversely to young tree-climbers, mature tree-climbing individuals barely produce thigmotropic C2 axes (figures 1*c* and 4*a*,*b*). Plants climbing on boulders produce shorter modules during the climbing phase, tightly following the boulders' uneven contour (figure 2*a*,*c*). Contrary to long-vertical climbers, main C1 axes of bouldering plants undergo a conspicuous change in their modules' growth behaviour from thigmotropic on the boulder surface to plagiotropic above the boulder (figure 2*a*,*b*). Owing to their thigmotropic growth behaviour, C2 axes are then restricted to a short basal climbing phase of bouldering plants (figures 2*a* and 4*c*,*d*). Bouldering plants are characterized by an increased production of orthotropic C3 axes which develop almost entirely from the self-supporting and plagiotropic portion of the main C1 axes (figures 2*a* and 4*c*,*d*). Leafy C4 axes are

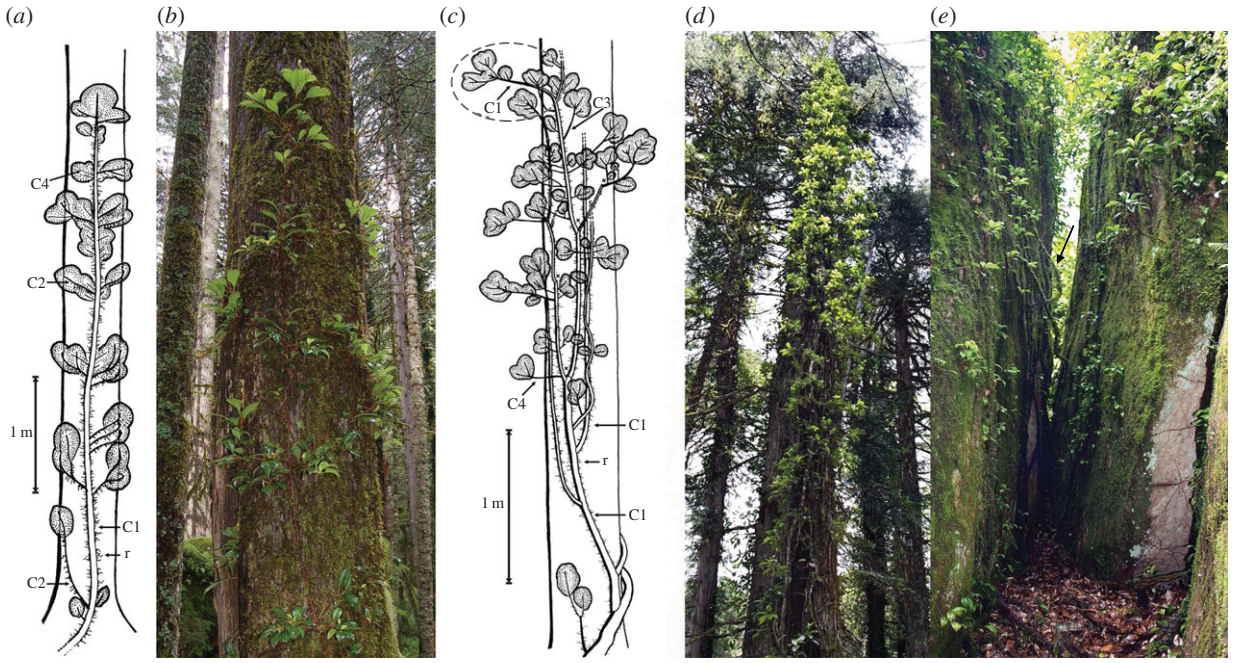

Figure 1. Long-vertical climbing habit of *H. seemannii*. Schematic representations and pictures of young (*a*,*b*) and mature tree (*c*,*d*) and rock wall (*e*) climbing individuals in their natural habitat. (*a*,*b*) Rhythmic branching is illustrated in the young individual by its regularly arranged lateral self-supporting axes departing from the main C1 climbing axis. (*c*) Change in growth direction is observed in C1 axes (top left C1 axis enclosed in grey dotted line), where apical portions lose contact with the support and laterally exhibit a self-supporting and plagiotropic segment. (*e*) Black arrow points to a main climbing C1 axis detached from the rock wall surface. Crosses indicate dead apices. Abbreviation r is for adhesive roots.

Figure 2. Bouldering habit of *H. seemannii*. (*a*–*c*) Schematic representations and pictures of a mature individual in its natural habitat. (*a*) The main C1 axis changes its growth direction after reaching the end of the supporting boulder (denoted by an asterisk). Several C3 orthotropic axes arise from the main C1 self-supporting axis portion, whereas C2 axes solely arise from the main C1 climbing axis portion. C4 axes are scarce and arise from the C1 main axis. (*c*) Main C1 axis exhibits short modules closely following the sinuous boulder's surface. Crosses indicate dead apices. Abbreviation r is for adhesive roots.

rather scarce and, if present, develop either from C1 or C3 axes (figures 2*a* and 4*c*,*d*).

3.2. Mechanical architecture

Hydrangea seemannii showed a general decrease in Young's modulus towards large-diameter segments with Young's modulus values ranging from *ca* 2300 to 560 MPa (figure 5). Segments belonging to the first developmental stage were characterized by a wide range of Young's modulus values (*ca* 820–2300 MPa) largely overlapping among axis types and highly varying within them (figure 5). Segments corresponding to the second developmental stage, almost entirely constituted by the main structural C1 axis, showed a marked decrease of Young's modulus from *ca* 1500–1400 MPa down to *ca* 560 MPa with increasing stem diameter (figure 5).

When considering the entire developmental range, both climbing phenotypes exhibited a general decrease in Young's modulus with increasing stem diameter; Young's modulus values ranged from *ca* 2300 to 560 MPa in tree-climbers and from *ca* 2300 to 820 MPa in boulder-climbers (figure 5). However, the nested ANOVA analysis showed no significant differences in Young's modulus between climbing phenotypes in neither of the two stem diameter classes and only lower *p*-values were retrieved for stem segments more than or equal to 7.4 mm (figure 6). Within the first developmental stage, tree-climbers showed a mean Young's modulus of 1432 MPa (s.d. $\pm$ 319) with Young's modulus values ranging from *ca* 2300 to 870 MPa, whereas rock-climbers had a mean Young's modulus of 1377 MPa (s.d. $\pm$ 332) with Young's modulus values ranging from *ca* 2300 to 820 MPa (figure 6). At the second developmental stage, Young's modulus values of tree-climbers ranged from *ca* 1970 to 560 MPa and showed a mean Young's modulus of 990 MPa (s.d. $\pm$ 419, figure 6). At this older developmental stage, boulder-climbers showed Young's modulus values only ranging from *ca* 1000 to 990 MPa with a mean Young's modulus of 1162 MPa (s.d. $\pm$ 215, figure 6).

Hydrangea sp. 1 exhibited a general decrease in Young's modulus throughout development, but a steep increase in Young's modulus for stem segments up to 10 mm in diameter with maximal Young's modulus values at around 2600 MPa (figure 5). Above 10 mm in diameter, a sudden decrease in Young's modulus occurred, followed by a more gradual

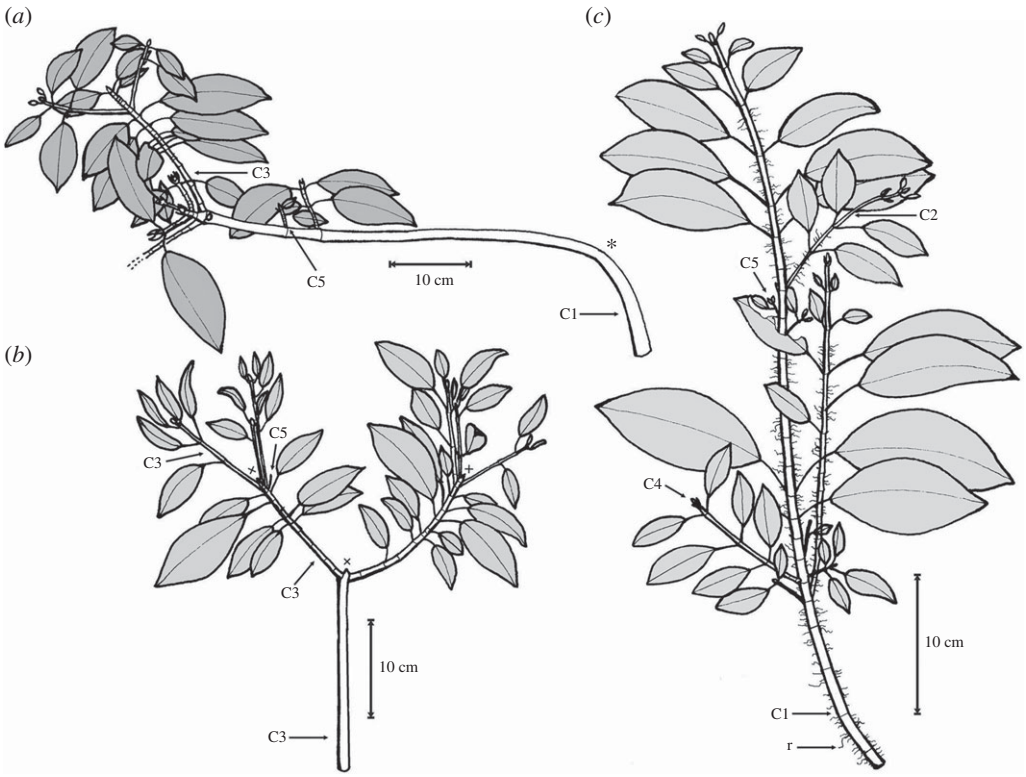

Figure 3. Detail of architectural axis categories in *H. seemannii*. (*a*) Axis category C1 forming the main axis of the plant structure. Here, the apical portion exhibits change in growth direction (denoted by an asterisk), loses contact with its support and bears C3 and C5 axis categories. (*b*) An orthotropic C3 axis forming forked partial reiterations and bearing a C5 axis. (*c*) Axis category C1, forming long running axes with adhesive roots and following the support. C1 axis category is here shown laterally bearing all other axis categories, except for C3. Crosses indicate dead apices. Abbreviation r is for adhesive roots.

decrease towards larger diameter segments (figure 5). The nested ANOVA analysis detected no significant differences in Young's modulus between *Hydrangea* sp. 1 and *H. seemannii* tree-climbers in neither of the younger ($p = 0.708$) nor older ($p = 0.846$) developmental stages. Similarly, no significant differences were obtained from the comparison of *Hydrangea* sp. 1 and *H. seemannii* boulder-climbers at the first developmental stage ($p = 0.846$) and lower, yet no significant *p*-values were retrieved in the older developmental stage ($p = 0.348$).

3.3. Stem anatomical organization and wood density

Considering the entire developmental range of *H. seemannii*, contribution to the total cross-sectional area and second moment of area showed a marked decrease for the parenchymatous pith, a slight decrease for the soft outer cortical tissues and a marked increase for the lignified pith along with wood towards larger diameter segments, whereas pith eccentricity markedly increased towards older developmental stages (see the electronic supplementary material, figure S2).

The nested ANOVA analysis showed no significant differences between climbing phenotypes for any of the measured stem tissue traits in either of the two developmental stages. Values of mean plus 2 s.d. overlapped to different degrees between climbing phenotypes. This was observed in all tissue organizations and in both stem developmental stages, with the exception of pith eccentricity at the older developmental stage (figure 7; electronic supplementary material, table S3). At this developmental stage, the mean of pith eccentricity plus 2 s.d. was higher in tree-climbers 0.37 (s.d. $\pm$ 0.15) than in boulder-climbers 0.09 (s.d. $\pm$ 0.07). At the second developmental stage, slightly higher contributions of lignified tissues and lower contributions of soft outer cortical tissues were observed in tree-climbers relative to boulder-climbers, however, this was not statistically supported (figure 7; electronic supplementary material, table S3).

Hydrangea seemannii tree-climbers showed significantly lower wood density than boulder-climbers for stems in the second developmental stage ($p = 0.016$; electronic supplementary material, table S3; figure 8). Tree-climbers showed a mean wood density of 539.24 (s.d. $\pm$ 16.40), whereas boulder-climbers had a mean wood density of 663.93 (s.d. $\pm$ 64.09, electronic supplementary material, table S3). Rings of eccentric climbing discs were narrower and compressed towards the abaxial position (the area close to the anchoring surface; figure 8), whereas rings of non-eccentric discs were more radially homogeneous (figure 8). Wider axes from both climbing phenotypes showed more dense wood around the pith periphery (figure 8).

4. Discussion

4.1. Do climbing phenotypes of *Hydrangea seemannii* differ in morphological architecture?

In cultivation, seedlings of *H. seemannii* quickly become unstable in the absence of support (M. Libert, Ghent University, Belgium, 2013, personal communication) and, in their natural habitat, fully self-supporting individuals have not been observed. This suggests that the ability to produce an early self-supporting phase characteristic of other climbers such as *M.* aff. *quinquepartita* [1] or the ability to develop fully self-supporting phenotypes such as in western poison oak (*Toxicodendron diversilobum* [9]) are not present in this species. Both climbing phenotypes of *H. seemannii* instead resemble more the general architectural model of the English

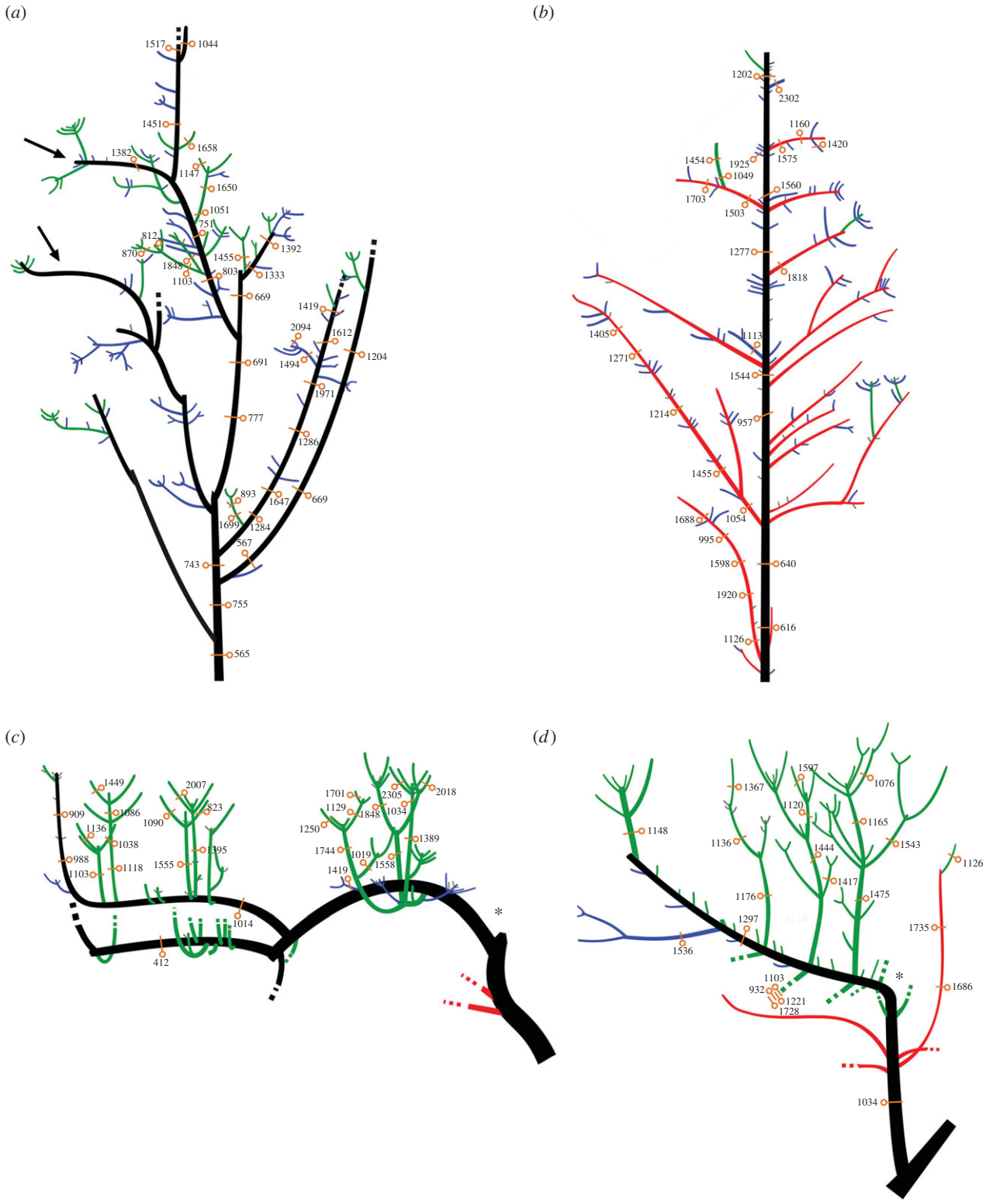

Figure 4. (*a*,*b*) Schematic of tree-climbers and (*c*,*d*) boulder-climbers morphological architecture. Black and red axes represent the structural supporting axes C1 and C2, whereas green and blue axes denote the resource acquisition axes C3 and C4. Numbers along branches of the schematic correspond to measurements of Young's modulus (MPa) graphed in figure 5. (Online version in colour.)

ivy (*H. helix*), having a plagiotropic juvenile form and later climbing by means of adhesive roots [12,30].

In contrast to the long internodes and relatively straight stems in tree-climbers of *H. seemannii*, boulder-climbers of the species showed shorter internodes and skewed stems, which might represent morphological plastic responses to different climbing environments such as light availability and surface contour. Compared with boulder surfaces, light incidence at tree surfaces is potentially lower due to additional shading produced by the host tree branches and leaves. Stem lengthening has been reported as a frequent shade avoidance response in plants [31–34], supporting the idea that in *H. seemannii* this plastic response is related to light availability. In contrast to woody-climbers, trees and shrubs develop comparatively shorter internode lengths [13], suggesting that the shorter internodes of boulder-climbers are a morphological plastic response tending towards the production of a more self-supporting architecture. Moreover, the shorter, more rounded form of

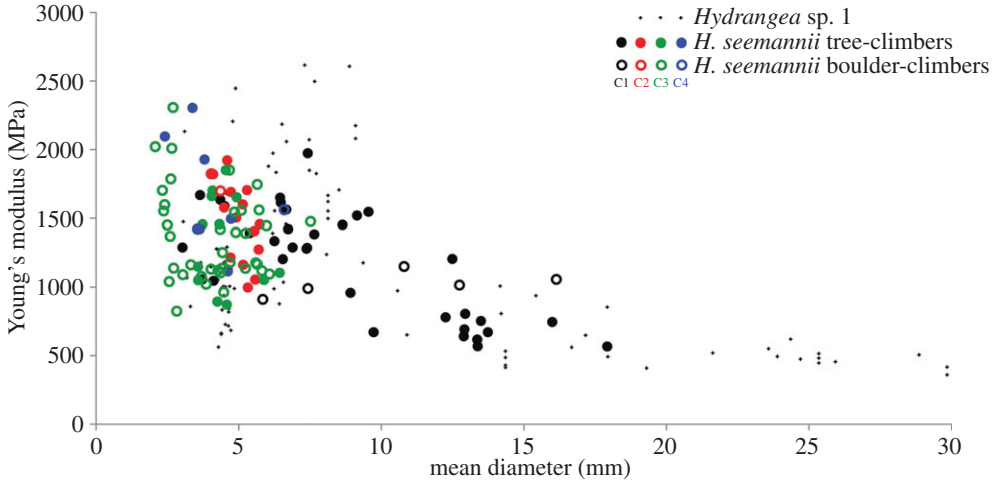

Figure 5. Mechanical properties of climbing phenotypes and their architectural axes. Young's modulus as a function of mean diameter. *Hydrangea seemannii* tree and boulder-climbers are denoted by solid and empty circles, respectively. *Hydrangea* sp. 1 is denoted by small black crosses. (Online version in colour.)

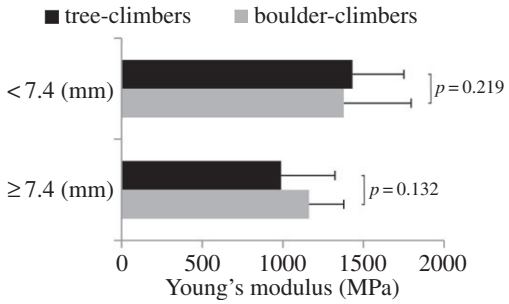

Figure 6. Mean Young's modulus and 1 s.d. of climbing phenotypes of *H. seemannii* in the two stem developmental stages.

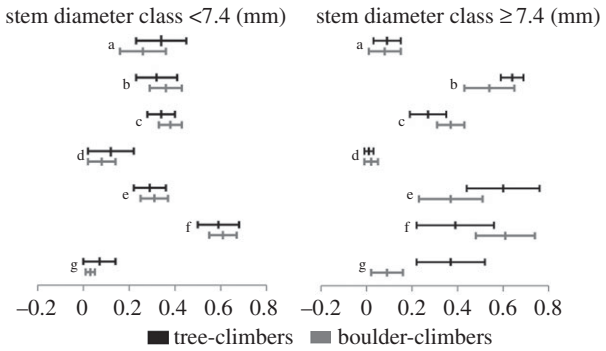

Figure 7. Mean and 2 s.d. of seven tissue organization traits of climbing phenotypes of *H. seemannii* in the two stem developmental stages. Parenchymatous pith, lignified pith along with wood and soft outer cortical tissues contribution to the total transverse-sectional area and second moment of area are denoted by (a–c) and (d–f), respectively, whereas (g) is for pith eccentricity.

the boulders in the vicinity substrate compared with the linear form of trees probably underline the differences in shape (askew versus straight stems) representing a plastic response to the climbing surface contour. This might be expected and a characteristic of climbing plants that attach closely to the host surface.

The different proportions of orthotropic-radial, C3 axes and plagiotropic-bilateral, C4 axes between climbing phenotypes of *H. seemannii* may be an indication of architectural plasticity linked to a leaf display optimization in each climbing environment. Above the boulder's surface, three-dimensional space is relatively open and light incidence is potentially higher than around vertical tree trunks. This possibly explains why boulder-climbers develop a high proportion of orthotropic-radial, C3 axes that can maximize leaf exposure and why tree-climbers develop more plagiotropic-bilateral C4 axes that can facilitate leaf displaying among the host branches and leaves. One consistent finding was that, in the more open apical parts of tree trunks, the proportion of orthotropic-radial C3 axes gradually increased. The increased production of orthotropic and radial axes in open environments has been previously reported in other woody growth forms [31,35], supporting the idea that these plastic responses in *H. seemannii* are linked to resource acquisition and influenced by three-dimensional space and light availability.

The conspicuous change in stem growth direction and habit found in boulder-climbers might represent a plastic response to support length variability. In contrast to tree trunks, boulders are comparatively shorter promoting an earlier loss of physical support and increased light exposure. In many climbing plants, when the tree canopy has successfully being reached, young self-supporting axes spread away from the support favouring leaf and flower display and facilitating the connection towards neighbouring supports [36]. The marked change in stem growth direction and habit of boulder-climbers might facilitate a faster display of leaf-bearing and fertile modules, therefore optimizing reproductive and resource acquisitioning in this particular climbing environment.

4.2. Do climbing phenotypes of *Hydrangea seemannii* differ in their mechanical architecture? And how do these climbing phenotypes differ from the strictly tree-climbing habit of *Hydrangea* sp. 1?

Self-supporting and non-self-supporting woody growth forms represent clearly different mechanical architectures; woody-climbers are generally characterized by a decrease in Young's modulus during development (e.g. with increasing stem diameter) and shrubs and trees show an opposite pattern with an increase in Young's modulus towards older developmental stages [4]. In our study, both boulder-climbers and tree-climbers of *H. seemannii* showed a general decrease in stiffness (i.e. Young's modulus) during development, which was similar

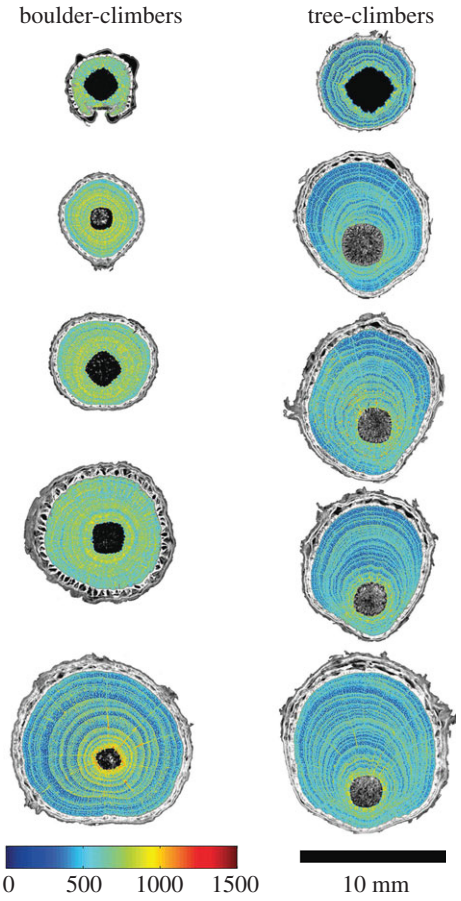

Figure 8. Comparative wood density between climbing phenotypes, wood density colour scale values are shown in kg m^{-3}. Pith and soft outer cortical tissues are shown in greyscale.

in magnitude to other climbing species with strong attachment systems (e.g. stem twiners and tendril climbers; [2]).

Despite the difference in climbing support (boulders versus trees), our analysis identified no significant differences in mechanical properties in either of the two developmental stages (figure 6). Somewhat different values were found for the second developmental stage; however, high standard deviations likely caused the observed differences in mean values to be not significant. These involved older stages of boulder-climbers that tended to have higher values of Young's modulus compared with older stages of tree-climbing stems. Several factors can explain the high variation in the obtained Young's modulus values. First, segments belonging to the same developmental stage showed contrasting axis habits exposing them to different mechanical forces. Second, segments of similar stem diameter showed variation in their secondary growth production, most likely as a response to the climbing surface heterogeneity, as well as to the axis habit, directly influencing the stem mechanical properties. Third, boulder and tree surfaces are non-uniform exposing the climbing segments to a number of different mechanical forces.

Overall, boulder-climbers appear to be slightly stiffer than tree-climbers at the old developmental stage. The potentially different values of old stems in boulder-climbers could represent a fine plastic adjustment to a completely static climbing surface. In turn, climbing on tree trunks may require more compliant stems in order to endure for instance the continuous wind-induced movements of the host plants, which specially affect climbing plants, such as root-climbers, that closely and strongly attach to their climbing surfaces. Moreover, different degrees of attachment might result from climbing on the relatively smooth boulder surface compared with the rough tree surface, resulting in a lower strength of attachment in boulder-climbers than in tree-climbers. As detailed above (see also [1,2]), firmly attached climbers generally develop more compliant tissues in older developmental stages than weakly attached ones; also believed to be helpful when handling the stress produced by close contact with the host.

When compared with the strictly tree-climbing species *Hydrangea* sp. 1, climbing phenotypes of *H. seemannii* showed no significant differences in either of the two developmental stages. However, lower *p*-values were obtained in the comparison with *H. seemannii* boulder-climbers for the second developmental stage. This suggests that boulder-climbers present a slight, yet non-significant, deviation from the 'typical' tree-climbing mechanical behaviour, showing a fine plastic adjustment in a developmental stage that is highly affected by the close contact with the climbing surface.

4.3. Do climbing phenotypes of *Hydrangea seemannii* differ in their stem anatomical properties?

Anatomical development of *H. seemannii* involved a decrease of mostly non-lignified tissues (i.e. parenchymatous pith and soft outer cortical tissues), but an increase in contributions of lignified tissues (i.e. lignified pith along with wood). With the exception of pith eccentricity, all measured anatomical variables presented highly overlapping mean plus 2 s.d. ranges, especially at the first developmental stage, explaining the non-significant differences between climbing phenotypes (see discussion above). In the case of pith eccentricity, the non-significant *p*-values in addition might be the result of the nested factor design of the ANOVA analyses, because exclusion of this factor (data not shown) resulted in significantly different mean pith eccentricity values.

Although not statistically supported, somewhat higher contributions of lignified tissues and lower contributions of soft outer cortical tissues were observed in tree-climbers relative to boulder-climbers at the second developmental stage. Wood produced by tree-climbers at this older stage was significantly less dense than that of boulder-climbers. Wood density is known to be affected by the size and number of vessels, as well as by fibre wall thickness and therefore influences both stem conductance and stem stiffness [37–39]. Less dense wood, resulting from an increased vessel area fraction, is associated with a potentially higher conductance efficiency [15,38] and more compliant tissues [37,39]. Developmental plasticity resulted in a somewhat greater area of less dense wood in stems of old developmental stages of tree-climbers. This is consistent with higher hydraulic conductance capacities, which is particularly advantageous when water must be drawn to provision aerial shoots high in the canopy. In terms of mechanical properties, the somewhat different proportions of wood area could play a role in homogenizing mechanical properties between climbing phenotypes, despite significant differences in wood density. Given a fixed wood density and stem diameter, a lower proportion of wood would contribute to more compliant stem segments when compared with segments with a greater proportion of wood. Boulder-climbers present more dense wood, however, their wood proportion within the stem is lower, whereas tree-climbers show a greater area of less dense wood, possibly explaining why differences in wood density do not result in

statistically significant mechanical differences. A greater area of less dense wood is consistent with the mechanical demands of the tree-climbing habit, where close contact with the host tree and branches and swaying movement in the wind require flexibility rather than stiffness. In turn, the developmental plastic response of boulder-climbers is to produce a smaller area of more dense wood in old stem developmental stages necessarily to maintain the structural stability of the self-supporting habit of its axes without compromising its hydraulic demands.

Hydrangea seemannii and other *Cornidia* species develop ring-porous wood [40]. Ring-porous wood in self-supporting trees is typically characterized by growth rings with wide vessels (lower density) in the early wood and fibres and more narrow vessels (higher density) in the late wood. In trees, ring-porous wood is largely restricted to northern temperate species and shows the largest increase in stem conductivity with stem size, as well as an efficient conducting network compared with diffuse-porous wood or coniferous wood [41]. Both climbing phenotypes of *H. seemannii* develop ring-porous wood; however, tree-climbers present areas of low-density wood away from the anchoring side of the stem and relatively more dense wood close to the supporting surface (tension wood). In turn, boulder-climbers showed a more homogeneous distribution of wood with mostly higher density. Correspondingly, tree-climbers appeared to have more eccentric piths compared with boulder-climbers, although this does not reach statistical support. In *H. seemannii*, pith eccentricity appears to result from the cambium varying its activity in relation to the contact with the climbing substrate. In terms of strength of attachment, the limited inner wood production in tree-climbers maintains adhesive roots close together assuring an appropriated anchoring strength—an increased production of wood on the inner surface against the tree would instead prise attached roots away causing anchorage failure. In terms of structural stability, differences in wood density distributions (detected via cross sections) might represent a developmental plastic response acting towards providing more structurally stable tissue on the anchoring side of climbing stems, albeit compromising partially stem conductivity.

4.4. Can we assume growth form plasticity in *Hydrangea seemannii* resulting from variability in support type?

For phenotypic plasticity to take place, individuals with the same genotype, e.g. belonging to the same species and population, should show different observable characteristics, which can range from morphological or physiological to developmental and behavioural properties as a response to different environmental conditions [7,42]. Nevertheless, plant–environment interactions can be both complex and numerous, producing a continuum of growth form variations, where an absolute 'description' or 'categorization' is often difficult to provide [1,16,18,25]. While some plant species such as poison oak [9] can be clear-cut examples of phenotypic plasticity producing two contrasting growth forms (lianas versus shrubs), other species within the continuum of growth form variations can present finer plastic responses within individual growth form types. We investigated three critical aspects defining woody plant growth form variability including their morphological, mechanical and anatomical architectures.

Some of the overall characteristics of *H. seemannii* might at first sight suggest an absence or rather limited developmental plasticity at the growth form level—(i) the lack of entirely self-supporting individuals, (ii) the early dependence on the climbing habit, (iii) the marked decrease of stem stiffness during development and (iv) the general decrease in contributions of non-lignified tissues and increase in contributions of lignified tissues. However, other finer scale characteristics sustain the notion of yet finer plastic responses to support variability within the woody climbing growth form in which boulder-climbers tend to show a developmental plasticity towards a more self-supporting growth form. These plastic responses represent alternative root-climbing strategies that are potentially optimized for different climbing substrates and environmental conditions. One aspect of phenotypic plasticity is phenotypic accommodation which produces a buffering effect maintaining critical relationships among varying components and lessening the negative effects of change [43,44]. Evidence for phenotypic accommodation in morphological, hydraulic and mechanical stem traits has been provided in previous studies on woody plants [43,45,46]. In *H. seemannii*, this buffering effect could for example involve the production of smaller cross-sectional areas of more dense wood in boulder-climbers versus more area of less dense wood in tree-climbers, to equilibrate the mechanical and hydraulic constraints derived from their particular climbing environments.

Appropriate characterization of phenotypic plasticity is fundamental to studies such as evolutionary and conservation biology [7,31,32,47–49]. It has been advocated that plasticity conferring adaptive flexibility can influence patterns of species diversification (see review by [7]). Growth form evolutionary studies should therefore consider potential plasticity in growth form characterizations, because it might be influenced by natural selection. Conservation programmes should not only aim to preserve plant species in terms of their genetic diversity, but also to maintain the environmental variation of their habitat. Being the only temperate *Cornidia* representative of the northern hemisphere, *H. seemannii* must be considered a priority for conservation programmes.

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References

1. Ménard L, McKey D, Rowe NP. 2009 Developmental plasticity and biomechanics of treelets and lianas in *Manihot* aff. *quinquepartita* (Euphorbiaceae): a branch-angle climber of French Guiana. *Ann. Bot.* **103**, 1249–1259. (doi:10.1093/aob/mcp078)
2. Rowe NP, Isnard S, Gallenmüller F, Speck T. 2006 Diversity of mechanical architectures in climbing plants: an ecological perspective. In *Ecology and biomechanics: a mechanical approach to the ecology of animals and plants* (eds A Herrel, T Speck, NP Rowe), pp. 35–59. Boca Raton, FL: CRC Press.
3. Rowe NP, Speck T. 1998 Biomechanics of plant growth forms: the trouble with fossil plants. *Rev. Palaeobot. Palynol.* **102**, 43–62. (doi:10.1016/S0034-6667(98)00013-X)
4. Speck T, Rowe NP, Civeyrel L, Claβen-Bockhoff R, Neinhuis C, Spatz C. 2004 The potential of plant biomechanics in functional biology and systematics. In *Deep morphology: towards a renaissance of morphology in plant systematics* (eds T Stuessy, F Hörandl, V Mayer), pp. 241–271. Königstein, Germany: Gantner.
5. Samain M-S, Wanke S, Goetghebeur P. 2010 Unraveling extensive paraphyly in the genus *Hydrangea* s.l. with implications for the systematics of tribe Hydrangeeae. *Syst. Bot.* **35**, 593–600. (doi:10.1600/036364410792495827)
6. Granados Mendoza C, Wanke S, Salomo K, Goetghebeur P, Samain M-S. 2013 Application of the phylogenetic informativeness method to chloroplast markers: a test case of closely related species in tribe Hydrangeeae (Hydrangeaceae). *Mol. Phylogenet. Evol.* **66**, 233–242. (doi:10.1016/j.ympev.2012.09.029)
7. Sultan SE. 2000 Phenotypic plasticity for plant development, function and life history. *Trends Plant Sci.* **5**, 537–542. (doi:10.1016/S1360-1385(00)01797-0)
8. Gallenmüller F, Müller U, Rowe NP, Speck T. 2001 The growth form of *Croton pullei* (Euphorbiaceae): functional morphology and biomechanics of a neotropical liana. *Plant Biol.* **3**, 50–61. (doi:10.1055/s-2001-11750)
9. Gartner BL. 1991 Structural stability and architecture of vines versus shrubs of poison oak, *Toxicodendron diversilobum*. *Ecology* **72**, 2005–2115. (doi:10.2307/1941555)
10. Gartner BL. 1991 Is the climbing habit of poison oak ecotypic? *Funct. Ecol.* **5**, 696–704. (doi:10.2307/2389490)
11. Arbonnier M. 2004 *Trees, shrubs and lianas of West African dry zones*, p. 574. Paris, France: CIRAD, Margraf Publishers Gmbh, MNHN.
12. Cremers G. 1973 Architecture de quelques lianes d'Afrique tropicale. *Candollea* **28**, 249–80.
13. Hallé F, Oldeman RAA, Tomlinson PB. 1978 *Tropical trees and forests: an architectural analysis*. Berlin, Germany: Springer.
14. Frenzke L, Wanke S, Isnard S, Stoll A, Neinhuis C, Rowe NP. 2011 Stem biomechanics of the giant moss *Dendroligotrichum dendroides* s.l. and its significance for growth form diversity in mosses. *J. Bryol.* **33**, 229–236. (doi:10.1179/1743282011Y.0000000011)
15. Isnard S, Speck T, Rowe NP. 2003 Mechanical architecture and development in *Clematis*: implications for canalised evolution of growth forms. *New Phytol.* **158**, 543–559. (doi:10.1046/j.1469-8137.2003.00771.x)
16. Lahaye R, Civeyrel L, Speck T, Rowe NP. 2005 Evolution of shrub-like growth forms in the lianoid subfamily Secamonoideae (Apocynaceae s.l.) of Madagascar: phylogeny, biomechanics, and development. *Am. J. Bot.* **92**, 1381–1396. (doi:10.3732/ajb.92.8.1381)
17. Niklas KJ. 1992 *Plant biomechanics: an engineering approach to plant form and function*, p. 607. Chicago, IL: University of Chicago Press.
18. Wagner ST, Isnard S, Rowe NP, Samain M-S, Neinhuis C, Wanke S. 2012 Escaping the lianoid habit: evolution of shrub-like growth forms in *Aristolochia* subgenus *Isotrema* (Aristolochiaceae). *Am. J. Bot.* **99**, 1609–1629. (doi:10.3732/ajb.1200244)
19. Barthélémy D, Caraglio Y. 2007 Plant architecture: a dynamic, multilevel and comprehensive approach to plant form, structure and ontogeny. *Ann. Bot.* **99**, 375–407. (doi:10.1093/aob/mcl260)
20. Stecconi M, Puntieri JG, Barthélémy D. 2010 An architectural approach to the growth forms of *Nothofagus pumilio* (Nothofagaceae) along an altitudinal gradient. *Botany* **88**, 699–709. (doi:10.1139/B10-040)
21. García-Arévalo A. 2008 Vegetación y flora de un bosque relictual de *Picea chihuahuana* Martínez del norte de México. *Polibotánica* **25**, 45–68.
22. Campos Villanueva Á, Lawrence MK, Delgado Salinas A. 2004 *Bejucos y otras trepadoras de la Estación de Biología Tropical Los Tuxtlas, Veracruz, México*. Mexico City, Mexico: UNAM, Instituto de Biología.
23. Granados Mendoza C, Ballesteros-Barrera C, Goetghebeur P, Samain M-S. 2010 Conservation status of *Hydrangea seemannii* (Hydrangeaceae) in Mexico: an inference based on ecological niche modelling. In *Scripta Botanica Belgica* (eds A Bogaerts, E Robbrecht, J Rammeloo), pp. 50. Meise, Belgium: National Botanic Garden of Belgium.
24. Vincent JF. 1990 *Structural biomaterials*. Princeton, NJ: Princeton University Press.
25. Isnard S *et al*. 2012 Growth form evolution in Piperales and its relevance for understanding angiosperm diversification: an integrative approach combining plant architecture, anatomy, and biomechanics. *Int. J. Plant Sci.* **173**, 610–639. (doi:10.1086/665821)
26. Rasband WS. 2012 *Image J*. Bethesda, MD: U.S. National Institutes of Health. See http://imagej.nih.gov/ij/.
27. Dierick M, Van Loo D, Masschaele B, Van den Bulcke J, Van Acker J, Cnudde V, Van Hoorebeke Ugent L. 2014 Recent scanner developments at UGCT. *Nucl. Instrum. Methods Phys. Res. B Beam Interact. Mater. Atoms* **324**, 35–40. (doi:10.1016/j.nimb.2013.10.051)
28. Masschaele BC, Cnudde V, Dierick M, Jacobs P, Van Hoorebeke L, Vlassenbroeck J. 2007 UGCT: new X-ray radiography and tomography facility. *Nucl. Instrum. Methods Phys. Res. A Accel. Spectrom. Detect. Assoc. Equip.* **580**, 266–269. (doi:10.1016/j.nima.2007.05.099)
29. De Ridder M *et al*. 2011 High-resolution proxies for wood density variations in *Terminalia superba*. *Ann. Bot.* **107**, 293–302. (doi:10.1093/aob/mcq224)
30. Cremers G. 1974 Architecture de quelques lianes d'Afrique tropicale. 2. *Candollea* **29**, 57–110.
31. Charles-Dominique T, Edelin C, Bouchard A. 2010 Architectural strategies of *Cornus sericea*, a native but invasive shrub of Southern Quebec, Canada, under an open or a closed canopy. *Ann. Bot.* **105**, 205–220. (doi:10.1093/aob/mcp273)
32. Charles-Dominique T, Edelin C, Brisson J, Bouchard A. 2012 Architectural strategies of *Rhamnus cathartica* (Rhamnaceae) in relation to canopy openness. *Botany* **90**, 976–989. (doi:10.1139/b2012-069)
33. Child R, Morgan DC, Smith H. 1981 Control of development in *Chenopodium album* L. by shadelight. *New Phytol.* **89**, 545–555. (doi:10.1111/j.1469-8137.1981.tb02334.x)
34. Gilbert IR, Jarvis PG, Smith H. 2001 Proximity signal and shade avoidance differences between early and late successional trees. *Nature* **411**, 792–795. (doi:10.1038/35081062)
35. King DA. 1998 Relationship between crown architecture and branch orientation in rain forest trees. *Ann. Bot.* **82**, 1–7. (doi:10.1006/anbo.1998.0638)
36. Isnard S, Silk WK. 2009 Moving with climbing plants from Charles Darwin's time into the 21st century. *Am. J. Bot.* **96**, 1205–1221. (doi:10.3732/ajb.0900045)
37. Rowe NP, Speck T. 2005 Plant growth forms: an ecological and evolutionary perspective. *New Phytol.* **166**, 61–72. (doi:10.1111/j.1469-8137.2004.01309.x)
38. Christensen-Dalsgaard KK *et al*. 2007 Changes in vessel anatomy in response to mechanical loading in six species of tropical trees. *New Phytol.* **176**, 610–622. (doi:10.1111/j.1469-8137.2007.02227.x)
39. Onoda Y, Richards AE, Westoby M. 2010 The relationship between stem biomechanics and wood density is modified by rainfall in 32 Australian woody plant species. *New Phytol.* **185**, 493–501. (doi:10.1111/j.1469-8137.2009.03088.x)
40. Gregory M. 2003 Hydrangeaceae. In *Anatomy of the dicotyledons* (eds DF Cutler, M Gregory), pp. 44–55. New York, NY: Oxford University Press.

41. McCulloh K, Sperry JS, Lachenbruch B, Meinzer FC, Reich PB, Voelker S. 2010 Moving water well: comparing hydraulic efficiency in twigs and trunks of coniferous, ring-porous, and diffuse-porous saplings from temperate and tropical forests. *New Phytol.* **186**, 439–450. (doi:10.1111/j.1469-8137.2010.03181.x)
42. Sultan SE. 2004 Promising directions in plant phenotypic plasticity. *Perspect. Plant. Ecol. Evol. Syst.* **6**, 227–233. (doi:10.1078/1433-8319-00082)
43. Olson ME, Aguirre-Hernández R, Rosell JA. 2009 Universal foliage–stem scaling across environments and species in dicot trees: plasticity, biomechanics and Corner's Rules. *Ecol. Lett.* **12**, 210–219. (doi:10.1111/j.1461-0248.2008.01275.x)
44. West-Eberhard MJ. 2005 Phenotypic accommodation: adaptive innovation due to developmental plasticity. *J. Exp. Zool. B Mol. Dev. Evol.* **304B**, 610–618. (doi:10.1002/jez.b.21071)
45. Rosell JA, Olson ME. 2007 Testing implicit assumptions regarding the age versus size dependence of stem biomechanics using *Pittocaulon* (*Senecio*) *praecox* (Asteraceae). *Am. J. Bot.* **94**, 161–172. (doi:10.3732/ajb.94.2.161)
46. Weitz JS, Ogle K, Horn HS. 2006 Ontogenetically stable hydraulic design in woody plants. *Funct. Ecol.* **20**, 191–199. (doi:10.1111/j.1365-2435.2006.01083.x)
47. Noel F, Machon N, Porcher E. 2007 No genetic diversity at molecular markers and strong phenotypic plasticity in populations of *Ranunculus nodiflorus*, an endangered plant species in France. *Ann. Bot.* **99**, 1203–1212. (doi:10.1093/aob/mcm067)
48. Körner C, Basler D. 2010 Phenology under global warming. *Science* **327**, 1461–1462. (doi:10.1126/science.1186473)
49. Nicotra AB *et al*. 2010 Plant phenotypic plasticity in a changing climate. *Trends Plant Sci.* **15**, 684–692. (doi:10.1016/j.tplants.2010.09.008)